

AMENDMENTS TO THE CLAIMS

1. (currently amended) A high throughput screening method for biological agents affecting fatty acid biosynthesis, wherein the method comprises:

(A) providing a reaction mixture comprising

- (1) (a) an acyl carrier moiety or (b) the enzymes and precursors precursors sufficient to generate the acyl carrier moiety;
- (2) a bacterial enzymatic pathway comprising at least two consecutively acting enzymes selected from the group consisting of:
 - (a) malonyl-CoA:ACP transacylase,
 - (b) β -ketoacyl-ACP synthase III,
 - (c) NADPH-dependent β -ketoacyl-ACP reductase,
 - (d) β -hydroxyacyl-ACP dehydrase, and
 - (e) enoyl-ACP reductase; and

(3) first substrates and cofactors required for the operation of the enzymes;

(B) contacting the reaction mixture with ~~the reaction mixture~~ a bioactive agent;

(C) conducting a high throughput measurement of the activity of the enzymatic pathway;
and

(D) determining if the contacting altered the activity of the enzymatic pathway.

2. (original) The high throughput screen of claim 1, wherein the bacterial enzymatic pathway comprises at least three consecutively acting enzymes selected from:

- (a) malonyl-CoA:ACP transacylase,
- (b) β -ketoacyl-ACP synthase III,
- (c) NADPH-dependent β -ketoacyl-ACP reductase,
- (d) β -hydroxyacyl-ACP dehydrase, and
- (e) enoyl-ACP reductase.

3. (original) The high throughput screen of claim 1, wherein the bacterial enzymatic pathway comprises at least four consecutively acting enzymes selected from:

- (a) malonyl-CoA:ACP transacylase,

- (b) β -ketoacyl-ACP synthase III,
- (c) NADPH-dependent β -ketoacyl-ACP reductase,
- (d) β -hydroxyacyl-ACP dehydrase, and
- (e) enoyl-ACP reductase.

4. (original) The high throughput screen of claim 1, wherein the bacterial enzymatic pathway comprises at least five consecutively acting enzymes selected from:

- (a) malonyl-CoA:ACP transacylase,
- (b) β -ketoacyl-ACP synthase III,
- (c) NADPH-dependent β -ketoacyl-ACP reductase,
- (d) β -hydroxyacyl-ACP dehydrase, and
- (e) enoyl-ACP reductase.

5. (original) The high throughput screen of claim 1, wherein the high throughput measurement measures the activity of enoyl-ACP reductase.

6. (original) The high throughput screen of claim 1, wherein the high throughput measurement comprises:

- (1) photometrically measuring the consumption of NADH; or
- (2) providing [³H]NADH as a cofactor to the enzymatic pathway and capturing a radioactive product on a support that provides a scintillant.

7. (original) The high throughput screen of claim 1, further comprising: providing intermediate substrates in the reacting step, wherein the intermediate substrates are not derived from said first substrates and are provided in an amount adapted to maintain such intermediate substrates at a concentration at least approaching the K_m of the respective enzyme that acts on the substrate during the assay timeframe.

8. (original) The high throughput screen of claim 1, further comprising: selecting at least one of the enzymes from the corresponding enzyme produced by *Staphylococcus aureous*, *Haemophilus influenzae* or *Streptococcus pneumoniae*.

9. (original) The high throughput screen of claim 8, further comprising: providing as the enoyl-ACP reductase a NADH-specific enoyl-ACP reductase.

10. (original) The high throughput screen of claim 9, further comprising: providing to the reacting step NADPH in a constant amount such that the NADH consumption by enoyl-ACP reductase (FabI) can be quantitated accurately and without interference, or an amount effective to reduce NADH consumption by more NADPH-dependent enzymes.

11. (original) The high throughput screen of claim 10, further comprising: providing to the reacting step an NADPH regenerating enzyme system.

12. (original) The high throughput screen of claim 8, further comprising: providing as the β -ketoacyl-ACP synthase III a β -ketoacyl-ACP synthase III derived from *E. coli* or *H. influenzae*.

13. (original) The high throughput screen of claim 1, wherein the malonyl-CoA:ACP transacylase is derived from *Streptococcus* or *Staphylococcus*, the β -ketoacyl-ACP synthase III is derived from *Streptococcus*, *Staphylococcus* or *Escherichia*, the NADPH-dependent β -ketoacyl-ACP reductase is derived from *Streptococcus* or *Staphylococcus*, the β -hydroxyacyl-ACP dehydrase is derived from *Streptococcus* or *Staphylococcus*, and the enoyl-ACP reductase is derived from *Staphylococcus* or *Escherichia*.

14. (original) The high throughput screen of claim 1, wherein the NADPH-dependent β -ketoacyl-ACP reductase is derived from *Streptococcus*, *Staphylococcus* or *Pseudomonas*.

15. (original) The high throughput screen of claim 1, further comprising: β -ketoacyl-ACP synthase II as part of the enzymatic pathway.

16. (original) The high throughput screening method of claim 1, further comprising: when a bioactive agent affecting the enzymatic pathway is identified, applying one or more deconvolution assays for determining which enzymes in the enzyme pathway are affected, said deconvolution assays comprising contacting the identified bioactive agent with (i) an enzyme in the enzymatic pathway or (ii) two or more, but less than all, enzymes acting sequentially in the enzymatic pathway.

17. (currently amended) A screening method for biological agents affecting fatty acid biosynthesis:

(A) providing a reaction mixture comprising

- (1) (a) an acyl carrier moiety or (b) the enzymes and precursors precursors sufficient to generate the acyl carrier moiety;
- (2) a bacterial enzymatic pathway comprising at least two consecutively acting enzymes selected from:

- (a) malonyl-CoA:ACP transacylase,
- (b) β -ketoacyl-ACP synthase III,
- (c) NADPH-dependent β -ketoacyl-ACP reductase,
- (d) β -hydroxyacyl-ACP dehydrase, and
- (e) enoyl-ACP reductase; and

(3) first substrates and cofactors required for the operation of the enzymes;

(B) contacting the reaction mixture with the reaction mixture a bioactive agent;

(C) conducting a high throughput measurement of the activity of the enzymatic pathway;

and determining if the contacting altered the activity of the enzymatic pathway[.],

wherein at least one of the following applies:

- (1) the enoyl-ACP reductase is a NADH-specific enoyl-ACP reductase; or
- (2) the β -ketoacyl-ACP synthase III is a β -ketoacyl-ACP synthase III derived from *E. coli*[.] or *H. influenzae*; or
- (3) NADPH is provided to the reacting step in a constant amount such that the NADH consumption by enoyl-ACP reductase (FabI) can be quantitated accurately and without interference, or an amount effective to reduce NADH consumption by more NADPH-dependent enzymes; or

(4) the NADPH-dependent β -ketoacyl-ACP reductase is derived from *Streptococci*,
Staphylococci or *Pseudomonas*.

18. (original) A method for attachment of a phosphopantetheinyl prosthetic group to apo-ACP comprising the steps of:

providing apo-ACP,

chemically adding a phosphopantetheinyl prosthetic group to said apo-ACP

19. (original) The method of claim 18 wherein said phosphopantetheinyl prosthetic group is added at a serine moiety in said apo-ACP.

20. (original) The method of claim 19 wherein said serine is Ser₃₇ and said apo-ACP is of *Escherichia coli* apo-ACP.